Use of autologous whole blood clot in the treatment of complex surgical wounds: a case series

Objective: Dehiscence and infection of hard-to-heal surgical wounds results in an increased risk of complications and mortality. A hard-to-heal surgical wound will present decreased levels of growth factors along with increased levels of debris and matrix metalloproteinases, resulting in the destruction of the extracellular matrix (ECM). ActiGraft (RedDress Ltd., Israel) is an autologous whole blood clot treatment, created at a point of care, to promote wound healing. We hereby present the efficacy of ActiGraft in a case series of hard-to-heal surgical wounds.
Method: A registry study of patients with surgical wounds was conducted in private clinics and hospitals across the US and Israel (NCT04699305). Autologous whole blood clot was created at point of care using the patient's own blood.
Results: A total of 14 patients took part in the study. Autologous whole blood clot treatment resulted in a mean percent wound area reduction of 72.33% at four weeks, with 33.33% of wounds achieving complete closure by week 4. At week 12, 78.54% of the wounds achieved complete closure.
Conclusion: Surgical wounds in patients with comorbidities may fail to initiate the natural wound healing mechanism which in turn may cause deterioration of the wound into a hard-to-heal stage. In this case series, autologous whole blood clot treatment was able to restore wound healing, avoiding the risk of infection and amputation of an affected limb. The properties of autologous whole blood clot as an ECM reduce the risk of infection, causing the wound to progress from the inflammatory phase to the proliferative phase. Autologous whole blood clot treatment in hard-to-heal surgical wounds was found to be an effective approach, reducing the risk of infection and promoting cell granulation, resulting in wound closure.
Declaration of interest: ActiGraft kits were supplied by RedDress Ltd., Israel, which had no input with regard to the direction of research or the conclusions drawn, which were solely those of the authors. The authors have no conflicts of interest to declare.

autologous whole blood clot • complete healing • extracellular matrix • growth factors • surgical wounds • wound • wound care • wound dressing • wound healing

Management of surgical wounds differs between patients, based on various factors that may lead to morbidities. Many surgical sites become infected, leading to complications that require longer periods of recovery, higher costs of treatment and a significantly reduced quality of life (QoL), ultimately leading to increased risk of mortality.[1] The adverse effects of hard-to-heal surgical wounds may outweigh the benefits gained from the original surgery. Healing of surgical wounds tends to be interrupted after discharge from hospital due to changes in maintenance and observation of the wound environment. A surgical site can be contaminated as a result of dehiscence of the surgical incision, which causes a risk to the patient.[2] Wound dehiscence usually occurs 4–14 days postoperatively, and most commonly between days 6 and 8.[3] Dehiscence may occur because of friction to the area, causing breakdown of the cutaneous and subcutaneous tissues surrounding the surgical area. Irritation may also be caused to the surgical environment due to adverse reactions from suture or staple material when attempting primary closure. The cutaneous tissue decreases in tensile strength and fibroblast concentration, causing the surgical incision to continue breaking down,-leading to an open wound.[4] With the constant distortion of the surgical site, the wound healing process becomes interrupted, resulting in a complex or chronic nature.

Lack of consistent and proper maintenance and treatment of the wound causes further deterioration to the surgical site, leading to hard-to-heal wound formation. Hard-to-heal circumstances occur if the wound environment becomes hypoxic, with increased levels of debris, matrix metalloproteinases (MMPs), proteases, and decreased levels of necessary growth factors. Destruction of the extracellular matrix (ECM) may cause deficient levels of growth factors, cytokines and mediators necessary for the healing of the skin. Adequate levels of growth factors, nutrients and organised mechanisms, i.e. homeostasis, inflammation, proliferation and remodelling, can assist in modifying the wound bed to one that may continue healing, by bringing the wound to the proliferative phase from the inflammatory phase.

Maxim Gurevich,[1] MD, Head of the Diabetic Foot Ulcer Unit; **Stephen M Heinz**,[2] MD, Emergency Medicine Physician; **Ruhama Fridman**,[3] RN, Deputy Director in the Nursing Unit; **Jessie Hawkins**,[4] PhD, Director of Clinical Research; **Chinenye D Wachuku**,[5] DPM, Resident Physician*
***Corresponding author email:** chichiwachuku@gmail.com
1 Diabetic Foot Clinic, Hillel Yaffe Hospital, Hadera, Israel. **2** Medical Center of Aurora, Aurora, CO, US. **3** Tel-Hashomer Hospital, Ramat Gan, Israel. **4** Franklin Health Research Center, Franklin, TN, US. **5** Hoboken University Medical Center, Hoboken, NJ, US.

ActiGraft (RedDress Ltd., Israel) is an autologous whole blood clot, created at the point of care to promote wound healing and granulation over skin defects, and it can take place in an outpatient setting. The blood clot is created from the patient's own peripheral blood and prepared into a whole blood clot matrix. The autologous whole blood clot incorporates itself into the surrounding tissue, encompassing any skin defects in the area to which it is applied.[5] The autologous whole blood clot supports wound haemostasis by taking the form of a blood clot which eliminates debris and drainage consisting of blood, purulence and serous fluid. The blood clot forms a matrix which is used as a scaffold to assist in the reconstruction of the ECM as a means of attracting and allowing adhesion of necessary growth factors, such as: platelet-derived growth factor (PDGF); fibroblast growth factors (FGF); epidermal growth factor (EGF); vascular endothelial growth factor (VEGF); insulin-like growth factor (IGF); and transforming growth factor (TGF), to enhance and accelerate the wound into subsequent phases of the wound healing process.[6] It has been shown that an exterior blood clot helps in controlling microbial infection. The fibrin in the clot plays a pivotal role in controlling infection at injury sites, forming a barrier that delays bacterial infiltration and movement into the wound, as well as slowing bacterial proliferation.[7]

The scaffold also assists in protecting the wound while the body removes debris from the wound bed, producing oxygenation and nutrients via angiogenesis of the wound bed through VEGF, and coalescence of wound edges through granulation during tissue regeneration in order to provide complete wound closure or closure of overexposed structures. Once in the proliferative phase, the scaffold recruits endothelial cells and fibroblasts to ultimately construct a permanent ECM. During this phase, a continuation of granulation occurs to fill skin defects and provide coalescence of cutaneous wound edges. Once this has been achieved, the blood clot dries and forms a scab, which then acts as a protective barrier by allowing the cutaneous tissue underneath to properly remodel without any interruption. Once the scab falls off, the previous wound bed is left with a collagenous scar.

Table 1. Patient demographics (n=14)

Characteristic	n	%
Sex		
Male	5	35.72%
Female	9	64.28%
Race		
White	14	100.0%

In this study, we aimed to explore the efficacy of autologous whole blood clot in achieving complete wound closure in hard-to-heal surgical wounds.

Method

Patient eligibility and enrolment

Study participants included wound care patients in hospitals and clinics across the US and Israel as part of a registry study (NCT04699305). Inclusion criteria were patients ≥18 years of age, with acute wounds or hard-to-heal surgical wounds. Patients or their legally authorised representatives gave written informed consent prior to the patient's participation in the study, and this covered all the patients' medical history, wound properties and wound images for the purpose of publication.

Autologous whole blood clot application and procedure

Blood was withdrawn from the patient into acid citrate dextrose adenine (ACDA) vacuum tubes (Sanwell, China). The blood was then gently mixed with a calcium coagulant and kaolin in a coagulation mould to create a blood clot, which took approximately eight minutes. The clot was then attached to the wound bed using steri-strips and was covered with a non-adherent dressing. A secondary hydrophilic foam dressing was used on top of the non-adherent pad. Re-application was performed weekly.

Statistical analysis

Demographic and patient history data were described with the mean and standard deviation (SD) for normally

Table 2. Wound characteristics

Case	Wound type	Wound duration	Previous treatments
1	Below-the-knee amputation	13 weeks	Surgical debridement, NPWT, hyperbaric oxygen
2	Post amputation of the great toe	>4 weeks	NPWT and advanced dressing
3	Transmetatarsal amputation	2 weeks	NPWT
4	Caesarean section	2 weeks	
5	Lower abdomen dehiscence	13 weeks	Debridement, collagenase ointment, foam dressings, advanced dressing
6	Abdomen	7 months	NPWT, surgical debridement, advanced dressing
7	Thigh dehiscence	3 years	NPWT, re-surgery, advanced dressing
8	Gunshot wound	10 days	Sharp debridement
9	Sacral skin flap dehiscence	8 months	Skin flap, surgical debridement, NPWT, skin graft, advanced dressing

NPWT—negative-pressure wound therapy

Fig 1. Case study 1. A 68-year-old female patient, who was wheelchair-bound, underwent a below-the-knee amputation of the left leg. Day 0: Fibrogranular wound bed with undermining and macerated wound edges. Wound bed measures approximately 10×5cm **(a)**. Week 8: after application of autologous whole blood clot, wound bed consists of granulation with decreased undermining and overall size of wound **(b)**

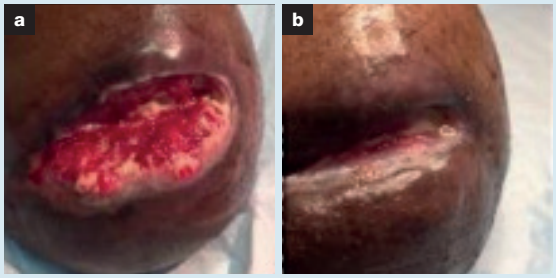


Fig 2. Case study 2. A 76-year-old male patient underwent an amputation of the great toe. Day 1: Extensive fibronecrotic tissue present in the wound bed measuring approximately 10×5cm with undermining **(a)**. Week 18: after autologous whole blood clot application, complete wound closure and coalescence of wound edges. Elimination of fibronecrotic tissue with increase in tissue granulation and epithelial growth of the skin **(b)**

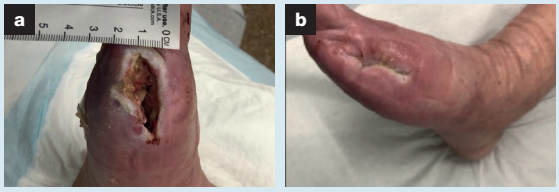
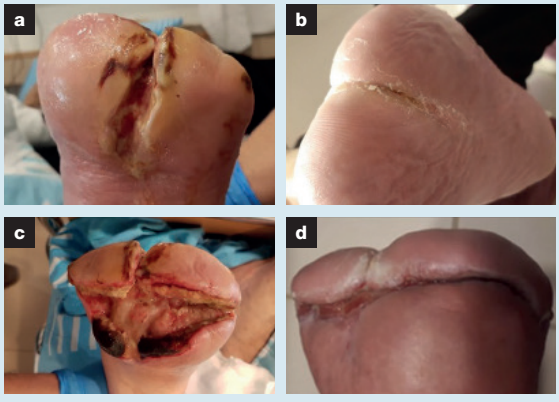


Fig 3. Case study 3. A 72-year-old female patient with multiple comorbidities underwent a transmetatarsal amputation of the left foot. Initial presentation as fibronecrotic wound with undermining and formation of an epibole **(a,c)**. After application of autologous whole blood clot over a 10-week period, complete closure and coalescence of wound edges **(b,d)**. Healthy granulation tissue observed with epithelial growth to enhance skin closure **(b,d)**



distributed continuous data, and the median and interquartile range (IQR) for non-normally distributed continuous data. Categorical data were described using frequencies and graphs. Assumptions of normality were evaluated using box plots, histograms and a Shapiro–Wilks normality test.

Results

A total of 14 patients were evaluated and nine had sufficient data to measure progress from baseline to week 4 (n=9). This population was 64.28% female (Table 1), with a mean age of 66.41±12.81 years (range: 33.2–81 years). Mean wound size at baseline was 21.47±19.30cm^2 (range: 1.7–57.0cm^2). The wounds had failed previous treatments prior to autologous whole blood clot application (Table 2). By week 4, the mean wound size was 6.52±7.63cm^2 (range: 0.0–22.0cm^2). It took 4.5±2.38 weeks for patients in this group to achieve complete healing (range: 3–8 weeks) with 6±3.61 (range: 3–13) applications of the autologous whole blood clot.

The percent wound area reduction (PAR) during the four-week period of treatment was 72.33±25.39%, (range: 25–100%; 95% confidence interval (CI): 52.82–91.85% area reduction). A total of three (33.33%) patients achieved complete healing by week 4, and eight (88.89%) patients experienced PAR at week 4 which exceeded 50%. A complete closure by week 12 was achieved in 78.54% of the treated wounds. Autologous whole blood clot treatment was found to have a high safety profile, with no adverse effects or complications reported among the patients.

Case study 1

A 68-year-old female patient, who was wheelchair-bound, underwent a below-the-knee amputation (BKA) of the left leg with a failure to muscle flap after significant destruction of the wound margins and a positive probe to bone result for suspected osteomyelitis. The patient's medical history included type 2 diabetes and peripheral vascular disease (PVD). Following the surgery, several treatments were given to increase re-epithelialisation and to close the surgical wound, including surgical debridement, hyperbaric oxygen, vacuum-assisted wound closure (VAC), and negative pressure wound therapy for a total of 13 weeks. The wound continued to deteriorate, leading to a division of the wound bed into two wounds due to epithelial bridging that separated it. The wound was treated with a total of five autologous whole blood clot applications, resulting in complete wound closure by week 8 (Fig 1).

Case study 2

A 76-year-old male patient underwent an amputation of the great toe. The patient had PVD, which was associated with a decrease in blood flow to the lower extremities and having a negative impact on wound healing. Following the amputation, the wound remained open without any signs of progression towards closure. The wound was treated with advanced

dressings and VAC therapy without any improvement. The wound bed comprised fibrotic tissue with minimal granulation evident, consistent with the finding of necrosis. Autologous whole blood clot application was introduced to the wound following a sharp debridement. In week 1, following the first application, the wound presented with a drastic reduction in size and increase in tissue granulation. A total of 10 autologous whole blood clot applications were made over 18 weeks to achieve complete closure of the wound (Fig 2).

Case study 3

A 72-year-old female patient with multiple comorbidities that included type 2 diabetes, osteoarthritis, osteoporosis, chronic renal failure, hypertension, artery disease, obesity, PVD and chronic obstructive pulmonary disease, underwent a transmetatarsal amputation of the left foot. Following the amputation, the wound failed to progress towards healing, resulting in wound dehiscence and an overall wound bed area of 37cm^2. Wound VAC treatment was applied with no improvement and a recommendation of a BKA was made. Autologous whole blood clot treatment was introduced to the wound as a last option before the BKA. Following autologous whole blood clot application, signs of tissue granulation were observed and an improvement in the wound was noted weekly until a complete closure was achieved. Debridement was also performed each week before applying a new clot. Autologous whole blood clot was applied nine times over 10 weeks and resulted in complete wound closure (Fig 3).

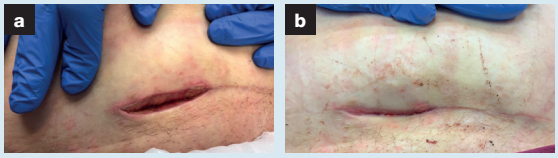
Case study 4

A 33-year-old female patient, having had a caesarean section, presented with a two-week-old, hard-to-heal surgical wound in the lower abdomen. The patient had a folic acid deficiency, iron deficiency, obesity, pemphigus vulgaris, chronic skin tag and fibromyositis. The patient was treated with a wound VAC, with no improvement. Autologous whole blood clot treatment was introduced once to the wound bed area resulting in complete wound closure on day 7 (Fig 4).

Discussion

Most wounds managed in an acute care setting are surgical. However, in patients with comorbidities, the wounds can be associated with a variety of complications, such as dehiscence, with surgical site infection being one of the most common complications.[8] The natural wound healing mechanism may fail in patients with comorbidities, delaying the progression of the wound from the inflammatory phase to the proliferative phase, and resulting in hard-to-heal wounds.[9] The condition of a hard-to-heal surgical wound results in a decrease in the patient's QoL, increased risk of infection, and wound deterioration leading to further medical intervention, prolonged hospitalisation periods, and increased burden on the health system.[10]

Fig 4. Case study 4. A 33-year-old female patient, having had a caesarean section, presented with a two-week-old, hard-to-heal surgical wound. Wound noted to the abdomen measuring approximately 8×3cm with a wound bed consisting of healthy granulation tissue **(a)**. After one application of autologous whole blood clot, wound closure is evident with the growth of granulation tissue extending from deep aspects to superficial aspects of the wound **(b)**



The wound healing cascade is an organised process, involving many cells in the area of the wound, enabling it to go through the four phases of wound healing: haemostasis, inflammation, proliferation and remodelling. Secretion of growth factors such as TGF-β, FGF, EGF, PDGF and VEGF takes place with integration into the wound bed. The attraction of neutrophils has a major role in reducing bacterial bioburden and introducing monocytes into the wound. This creates homeostasis in the wound area and promotes wound healing.[11–13]

The most common wound complication is the elevation of the inflammatory response, especially macrophages. Simultaneously, increased secretion of MMPs occurs, causing a breakdown in the ECM. This results in inhibition of the PDGF, TGF-β and VEGF secretion, preventing the wound from progressing to the proliferative phase.[14] Macrophages play a major role in wound healing by secreting pro-inflammatory cytokines that are necessary for the initiation of the inflammatory phase. This is mediated by M1 macrophages. However, progression towards the proliferative phase is a result of the transition of M1 macrophages into M2 anti-inflammatory macrophages. In hard-to-heal wounds, this transition fails, maintaining the wound in the inflammatory and hard-to-heal phase.[15]

In this study, the autologous whole blood clot, created in a point-of-care setting, was found to be safe and effective in treating hard-to-heal wounds of the lower extremities.[5,16] The autologous whole blood clot creates a scaffold that acts as an ECM, reestablishing the communication between the cells in the wound environment.[17] The ECM contains multiple noncellular scaffold proteins (i.e., collagen, elastin, fibronectin and proteoglycans), glycosaminoglycans, polysaccharides and water, which provide not only physical infrastructure but also act as a regulator of cellular activity with the cells in the wound area. The autologous whole blood clot was suggested to influence the transition of macrophages from an M1 to M2 characteristic, reducing MMP levels in the wound area and causing the wound to progress toward healing. The

Reflective questions

- What types of growth factor does the autologous whole blood clot attract to the wound area?
- What is the effect of the autologous whole blood clot on the extracellular matrix?
- What is the effect of the autologous whole blood clot on the wound's microbial bioburden?

autologous whole blood clot was found to lower bacterial bioburden by creating a barrier that prevents bacterial infiltration into the wound. Moreover, a plethora of evidence suggests that proteins and peptides in the ECM can trigger antibacterial activity both in vitro and in vivo, and the ECM is considered an ideal tissue to prevent infection.[18–20]

It is suggested that the autologous whole blood clot, by mimicking the ECM properties, adapts to the antibacterial activity of the ECM, lowering the risk of infection. The autologous whole blood clot not only advances the wound towards closure but also assists in preventing recurrent wound infections.

Limitations

This study identifies the effect of an autologous whole blot clot treatment using real-world effectiveness data. There is a need to conduct a large study with more patients to evaluate the statistically significant efficacy of the autologous whole blood clot in surgical cases. Moreover, the registry only included patients treated with the autologous whole blood clot and comparison group data were not available.

One benefit of a registry study is that it includes patients who are not typically included in clinical trials. These patients often have larger wounds, hard-to-heal wounds, or wounds with a long history. Thus, registry studies are likely to produce estimates of effectiveness that are lower than estimates of efficacy identified in controlled trials. A comparison of treatments with the autologous whole blood clot in patients with large, hard-to-heal wounds would likely produce a larger effect size.

Conclusion

The autologous whole blood clot used in this study was found to be safe and effective in treating hard-to-heal wounds of the lower extremity, causing the wound to progress from a hard-to-heal inflammatory phase to the proliferative phase of the wound healing cascade. The autologous whole blood clot promotes cell granulation and has been shown to have a high benefit in surgical wounds, not only by initiating the natural wound healing properties but also by preventing wound infection, reducing the risk for any postoperative complications. Autologous whole blood clot as a point-of-care treatment has the potential to reduce hospitalisation time, lowering the burden on the health system and, as an autologous treatment, showing a high safety pattern, reducing any negative implications that may arise due to immunological rejection. In conclusion, autologous whole blood clot treatment showed high efficacy in promoting delayed wound healing, contributing to the improvement of the patient's QoL. **JWC**

References

1 Britteon P, Cullum N, Sutton M. Association between psychological health and wound complications after surgery. Br J Surg 2017; 104(6):769–776. https://doi.org/10.1002/bjs.10474
2 Aksamija G, Mulabdic G, Rasic I, Aksamija L. Evaluation of risk factors of surgical wound dehiscence in adults after laparotomy. Med Arch 2016; 70(5):369–372. https://doi.org/10.5455/medarh.2016.70.369-372
3 Chun JJ, Yoon SM, Song WJ et al. Causes of surgical wound dehiscence: a multicenter study. J Wound Manag Res 2018; 14(2):74–79. https://doi.org/10.22467/jwmr.2018.00374
4 Cöl C, Soran A, Cöl M. Can postoperative abdominal wound dehiscence be predicted? Tokai J Exp Clin Med 1998; 23(3):123–127
5 Kushnir I, Kushnir A, Serena TE, Garfinkel D. Efficacy and safety of a novel autologous wound matrix in the management of complicated, chronic wounds: a pilot study. Wounds 2016; 28(9):317–327
6 Xue M, Jackson CJ. Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. Adv Wound Care 2015; 4(3):119–136. https://doi.org/10.1089/wound.2013.0485
7 Macrae FL, Duval C, Papareddy P et al. A fibrin biofilm covers blood clots and protects from microbial invasion. J Clin Invest 2018; 128(8):3356–3368. https://doi.org/10.1172/JCI98734
8 Allegranzi B, Nejad SB, Combescure C et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet 2011; 377(9761):228–241. https://doi.org/10.1016/S0140-6736(10)61458-4
9 Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature 2008; 453(7193):314–321. https://doi.org/10.1038/nature07039
10 Harding KG, Morris HL, Patel GK. Science, medicine, and the future: Healing chronic wounds. BMJ 2002; 324(7330):160–163. https://doi.org/10.1136/bmj.324.7330.160
11 Braund R, Hook S, Medlicott NJ. The role of topical growth factors in chronic wounds. Curr Drug Deliv 2007; 4(3):195–204. https://doi.org/10.2174/156720107781023857
12 Lawrence WT. Physiology of the acute wound. Clin Plast Surg 1998; 25(3):321–340. https://doi.org/10.1016/S0094-1298(20)32467-6
13 Kiritsy CP, Lynch SE. Role of growth factors in cutaneous wound healing: a review. Crit Rev Oral Biol Med 1993; 4(5):729–760. https://doi.org/10.1177/10454411930040050401
14 Caley MP, Martins VL, O'Toole EA. Metalloproteinases and wound healing. Adv Wound Care 2015; 4(4):225–234. https://doi.org/10.1089/wound.2014.0581
15 Snyder RJ, Schultz G, Wachuku C et al. Proposed mechanism of action of topically applied autologous blood clot tissue: a quintessential cellular and tissue based therapy. J Am Podiatr Med Assoc 2020; 13. https://doi.org/10.7547/20-140
16 Snyder RJ, Kasper MA, Patel K et al. Safety and efficacy of an autologous blood clot product in the management of Texas 1A or 2A neuropathic diabetic foot ulcers: a prospective, multicenter, open label pilot study. Wounds 2018; 30(7):84–89
17 Wong VW, Gurtner GC, Longaker MT. Wound healing: a paradigm for regeneration. Mayo Clin Proc 2013; 88(9):1022–1031. https://doi.org/10.1016/j.mayocp.2013.04.012
18 Sarikaya A, Record R, Wu CC et al. Antimicrobial activity associated with extracellular matrices. Tissue Eng 2002; 8(1):63–71. https://doi.org/10.1089/107632702753503063
19 Brennan EP, Reing J, Chew D et al. Antibacterial activity within degradation products of biological scaffolds composed of extracellular matrix. Tissue Eng 2006; 12(10):2949–2955. https://doi.org/10.1089/ten.2006.12.2949
20 Jiménez-Gastélum GR, Aguilar-Medina EM, Soto-Sainz E et al. Antimicrobial properties of extracellular matrix scaffolds for tissue engineering. BioMed Res Int 2019; 2019:1–7. https://doi.org/10.1155/2019/9641456